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# **EUROPEAN PATENT APPLICATION**

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Enzyme modified protein and process for its production.

A method of preparing a vegetable protein product having excellent solubility in aqueous solutions is disclosed. The product exhibits a greatly improved solubility and other functional properties over known materials and can be incorporated in a wide variety of food materials to form superior protein fortified foods. The process involves subjecting aqueous protein material to a controlled deamidation by a proteolytic enzyme under carefully controlled pH to hydrolyze and modify the protein, and heating the protein to inactivate the enzyme and stop the reaction. The aqueous protein can be dried and a dry, powdered protein product is recovered which is highly soluble in water, to the extent that substantially all of the modified protein may be rendered soluble in aqueous systems, if desired. This solubility increase is accomplished without excessive hydrolysis of the protein.

The present invention relates to a process for the preparation of vegetable protein food products of controlled solubility and, more particularly, to a process for preparing a vegetable protein food product, especially a soy protein food product, of high solubility. The food products prepared by the process of this invention will generally have a high solubility and very little bitter or beany flavour, and will generally exhibit improved functional properties over previously known materials. These properties may be achieved without harsh chemical treatment or excessive hydrolysis, which could otherwise significantly reduce the functional properties of the protein.

The preparation and modification of vegetable protein products by a variety of processes is known, including the production of protein products from soy protein, as is the treatment of such products to improve various of their characteristics, for example as disclosed in US A-3 642 490, US A-3 694 221 and US A-2 232 052. By way of example, enzymatic processes for obtaining food materials from vegetable protein materials are well known; a typical process is disclosed in US A-2 232 052. The technique most commonly employed in enzymatic processes requires a lengthy reaction time, typically several hours, which has to be at a pH optimum for the enzyme used, and is most commonly conducted as a batch type operation. However, conventional processes have not conducted such hydrolysis under pH and other conditions which are effective to expose the core proteins of the protein substrate to hydrolysis. We have now discovered that treatment under conditions which do expose and treat the core proteins is necessary if, as is desirable, solubility and organoleptic qualities are to be maximised. The process of this invention, which may be a semi-continuous type of operation or batch, uses a controlled pH with an enzymatic reaction partially to hydrolyze, deamidate and modify the protein reactant. The process of the present invention is capable of producing a product of unexpectedly superior functional properties and with a solubility which may be substantially 100%.

Prior processes of hydrolysing and/or deamidating vegetable protein materials, whilst producing a commercially useful product, have not been able to achieve solubilities above about 50 to 60% of the available protein since the core proteins of the vegetable protein material have been essentially untouched by the enzymatic action. Attempts to increase the solubility of the protein product, by increasing the harshness of the treatment, have not succeeded in increasing solubility, rather, instead, the nutritional and other functional properties of the protein were reduced.

We have now discovered an enzymatic method of processing vegetable protein, and particularly soybean protein materials, to form a desirably edible protein product that has the high solubility necessary to be functional but which has excellent foam and emulsion functionality.

By careful control of the process conditions, it is possible to obtain such a product which is also highly functional due to a controlled high solubility that enables excellent grade aqueous suspensions to be formed which exhibit excellent texture and are smooth, not chalky, lumpy or granular.

Thus, the present invention consists in a method of treating a protein material having an insoluble fraction, which comprises subjecting an aqueous slurry of the protein material to controlled hydrolysis and deamidation, the controlled hydrolysis and deamidation including adjusting the slurry to an elevated pH, adding a proteolytic enzyme to the slurry and reacting the slurry for a time and at a temperature which, in combination with the pH level and enzyme, are effective partially to hydrolyse and partially to deamidate the protein, the treatment being effective to substantially uniformly hydrolyse and deamidate across substantially the entire protein substrate including the insoluble fraction of the protein substrate, especially the core protein, thereby increasing the solubility of the protein and increasing the functional properties of the protein without producing a bitter flavour in the treated protein.

The product of the present invention can be used directly as a food and also will blend smoothly with products containing other flavourings without altering the character of the other flavourings.

The basic unique product obtained by the process of the present invention is one of excellent functionality and exceptional solubility. It is treated with a process involving heat, pH control and enzymatic hydrolysis. The product uniquely has an optimum combination of properties not obtainable heretofore in having a high solubility, which may be above 50 or even above 60% and up to about 100% for a product formed from an isolated protein. Hence, it can be used as a food product in various forms, with a high protein content. It also can be selectively added to a wide variety of other food products to achieve special results, for example, it may be used as a protein fortifier which is added to an aqueous solution by the consumer. When added to cold milk, the material of the present invention exhibits excellent solubility characteristics, which may be essentially 100%. The mouthfeel and taste of the milk mixture containing the product of the present invention is excellent and it can provide a refreshing and nutritious drink, for example, of the instant breakfast type.

The process of the present invention preferably treats the protein material as an aqueous slurry with controlled, elevated pH and enzyme treatment at an elevated temperature. The pH chosen, in combination

with the enzyme, makes the entire protein substrate available for enzyme hydrolysis and deamidation, including the core proteins present in the substrate. By careful regulation of the process, the degree of both solubility and deamidation of the resulting product can be controlled over a very wide range. The conditions of pH and temperature are chosen to prevent formation of LAL compounds (i.e. lysinal alanine compounds), for example less than about 300 ppm.

This invention was conceived and developed largely for soy protein materials because of the special problems encountered with such materials and because of their wide availability and reliable quality, which make them especially suitable for use as food materials. Therefore, it will be explained largely with respect to soy materials and has special application to such materials. However, it can be used for other protein materials in the broader aspects of the invention, and, in general, any edible protein material can be treated by the process of the present invention, for example, other vegetable (especially oilseed) materials, fish protein materials, microbial protein products or even, if desired, protein products derived from meat may be

The acceptance of vegetable protein materials, such as soybeans, peanuts, safflower, cottonseeds, sesame seeds, sunflower seeds, rapeseeds and others (any of which may be used in the process of the present invention), depends on modification of these materials to improve their functional properties. Extensive research has been conducted on these in an effort to develop useful food products. As a result, some of these materials are presently being processed to produce food products commonly called edible vegetable proteins. However, the functional properties, such as taste and solubility, and foam and emulsion forming properties of these materials is not satisfactory for many commercial food uses. The process of the present invention enables one to produce a novel protein material which has greatly improved solubility, without introduction of bitter flavours. Solubility can be as high as 100%. This result is achieved by an enzyme hydrolysis and partial deamidation reaction over substantially the entire protein substrate. Deamidation levels can preferably be varied between about 5 to 48%. The hydrolysis is preferably accomplished under ambient to elevated temperature conditions and under a controlled elevated pH which is effective to render the protein substrate soluble, including the core protein fractions, without degradation or excessive hydrolysis of the protein substrate, and in particular without excessive hydrolysis or degradation of the noncore fraction of the substrate.

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The process of the present invention may be applied to the treatment of any protein materials, especially vegetable protein materials, regardless of the concentration of protein therein. However, it is preferably applied to the treatment of vegetable and other protein concentrates, and most especially of vegetable and other protein isolates, preferably soy protein materials, especially soy protein isolates.

It is a further advantage of the process of the present invention that the solubility of the product may be broadened to encompass a wide pH range. When the hydrolysis reaction of the present invention has progressed to the desired state, the reaction may be stopped by inactivating the enzyme, for example by heat, as is well known in the art. The conditions of the process are preferably controlled to minimize or prevent the formation of undesirable LAL compounds, in particular by maintaining the pH of the reaction below about 12.

Generally, the pH of the process is controlled to between about 9 to 11 during the process. These limits have been found to be effective. A preferred pH of about 10 has been found to be particularly effective. Control of the pH may be by use of conventional food grade bases and buffers, such as sodium hydroxide, sodium bicarbonate, ammonium carbonate, sodium tripolyphosphates, hydrochloric acid and other conventional reagents. The optimum pH may differ somewhat for each particular enzyme used, but the process is generally effective within this pH range. The temperature of the reaction may preferably vary from about 10 °C, e.g. room temperature, to about 75 °C, and the process is also generally effective within this temperature range. The optimum temperature conditions may also vary somewhat, preferably within this range, for any particular system. Likewise the reaction time may also preferably vary within an effective range of from about 10 minutes to 4 hours (240 minutes) and may have different optimum values for a particular system of time, temperature, pH and particular enzyme, within the effective range.

The enzymes which are effective in the process of the present invention are generally those proteolytic enzymes which are commonly used for the hydrolysis of proteins and may be obtained from animal, plant and microbial sources. A variety of enzymes have proven satisfactory. These include papain, trypsin, ficin and a variety of bacterial and fungal proteases. The only limitation on the protease is that it be stable and not be inactivated by the pH used for the process.

The enzyme material may preferably be added at a preferred level of from about 0.01 to 5.0% by weight of protein material (dry basis), depending on the temperature and time conditions employed, the activity of the enzymes, and the degree of hydrolysis desired. (Enzyme activity may be defined as the amount of enzyme required to produce a standard amount of tyrosine from casein and maltose from starch

under standard conditions.) Levels of enzyme above 1.0% by weight may be used, but the cost may become prohibitive for some enzyme materials. At levels below 0.05% the enzymatic reaction may proceed too slowly to be commercially viable with some enzyme systems.

The treated protein slurry and enzyme reactant mass are typically and preferably reacted in a holding tank to provide a sufficient time lag for the reaction to proceed substantially to completion. When the enzymatic reaction has proceeded for a sufficient length of time, the reaction is stopped, e.g. by heating the slurry to a temperature sufficient to inactivate the enzymes. Temperatures above about 180°F (82°C) are normally sufficient to inactivate the enzyme activity. The heating step can be accomplished by passing the reacted material through a heat exchanger, by a jet cooking step, or by most conventional heating operations. Following inactivation, the pH of the product can be adjusted to a desired level or the pH can be adjusted prior to inactivation.

The treated protein preferably has a molecular weight (Mn) of from 800 to 4000.

The resulting slurried product can be used directly for food products. It is an attractive light coloured product. Alternatively it can be dried, with the dried product having excellent solubility in an aqueous medium.

If the slurry is dried, it is preferably flash dried because of the uniform fine, powdered product obtained, the economical continuous processing afforded thereby, and the excellent solubility characteristics of the powder. Of the flash drying techniques, spray drying is usually used. The product may be freeze dried, but this is more costly. The dried powder is capable of rapid simple rehydration to form a suspension simply by adding water and stirring, because of its high solubility. The product may be used as a substitute for dairy product derivatives, even dried skimmed milk, for a variety of purposes. The product has an excellent smooth texture and taste. The product stays in solution and does not settle out at the bottom of the container when mixed in an aqueous suspension.

The invention is further illustrated by the following non-limiting Examples.

## **EXAMPLE 1**

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860 pounds (390 kg) of isolated soy protein aqueous curd having a solids content of 14% by weight, was treated by adjusting the pH to a value of 8.0 using a 50% w/v aqueous solution of sodium hydroxide and was heated to 305°F (152°C) using a jet cooker. The duration of heating was about 9 seconds. Following this heat treatment the curd was cooled to 104°F (40°C). The cooled curd was adjusted to a pH value of 10 by the addition of a 50% w/v aqueous solution of sodium hydroxide, whilst rapidly stirring. 1.5% papain (Amano) based on the dry weight of the protein was added as a 10% aqueous slurry and was mixed with rapid stirring. The mixture was then reacted for 60 minutes while maintaining the pH at a value of 10 by the addition of a 50% w/v aqueous solution of sodium hydroxide. The reacted mixture was then adjusted to a pH value of 7. 5 - 7. 9 by the addition of concentrated aqueous hydrochloric acid and heated to inactivate the enzyme. Inactivation was accomplished by heating the mixture to 305°F (152°C). The duration of heating was about 9 seconds and the total time required to inactivate the reaction mixture was about 43 minutes. The inactivated mixture was then spray dried. The properties of the spray dried material are reported in Table 1.

## **EXAMPLE 2**

The jet cooked curd of Example 1 was cooled to 114°F (46°C), was adjusted to pH 10 and was treated with 2.0% pancreatin (Rohm COROLASE®), on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 37 minutes reaction time for the mixture. The pH of the reaction mixture was then adjusted to a value of 7. 5, after which the product was heat inactivated and spray dried as described in Example 1. The product properties are reported in Table 1.

# EXAMPLE 3

The jet cooked curd of Example 1 was cooled to 114°F (46°C), adjusted to a pH value of 10 by the addition of a 50% w/v aqueous solution of sodium hydroxide, and treated with 5% alcalase 2. 4 L, (Novo Laboratories), on a dry curd basis. The mixture was then reacted as described in Example 1. The pH was maintained at a value of 10 during the 82 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to a value of 7. 5, after which the product was heat inactivated and spray dried as described in Example 1. The product properties are reported in Table 1.

# **EXAMPLE 4**

The room temperature curd of Example 1 was adjusted to a pH value of 10 and treated with 1. 5% papain, (Amano), on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 52 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to a value of 7. 5, after which the product was heat inactivated and spray dried as described in Example 1. The product properties are reported in Table 1.

# **EXAMPLE 5**

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The room temperature curd of Example 1 was cooled to 10°C, adjusted to pH 10 and treated with 1. 5% pancreatin, (Rohm COROLASE®), on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 60 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to a value of 7. 5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

### **EXAMPLE 6**

The room temperature curd of Example 1 was adjusted to a pH value of 10 and treated with 0. 6% alcalase 2. 4 L, (Novo Laboratories), on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 20 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to a value of 7. 5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

### EXAMPLE 7

The room temperature curd of Example 1 was adjusted to a pH value of 10 and treated with 0. 25% papain, on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 90 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to 7. 5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

#### **EXAMPLE 8**

The room temperature curd of Example 1 was adjusted to a pH value of 9 and treated with 1.0% papain, on a dry curd basis, as described in Example 1. The pH was maintained at a value of 9 during the 90 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to 7. 5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

## **EXAMPLE 9**

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The room temperature curd of Example 1 was adjusted to a pH value of 11 and treated with 1.0% papain, on a dry curd basis, as described in Example 1. The pH was maintained at a value of 11 during the 90 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to 7.5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

## **EXAMPLE 10**

The room temperature curd of Example 1 was heated to 148°F (65°C), adjusted to a pH value of 10 and treated with 1.0% papain, on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 45 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to 7. 5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

## EXAMPLE 11

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The room temperature curd of Example 1 was heated to 194°F (90°C), for 5 minutes, cooled to room temperature, adjusted to a pH value of 10 and treated with 0. 25% trypsin (Sigma T8253), on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 30 minute reaction

time for the mixture. The pH of the reaction mixture was then adjusted to 7. 5, after which the product was heaf inactivated and freeze dried. The product properties are reported in Table 1.

# **EXAMPLE 12**

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The jet cooked curd of Example 1 was cooled to room temperature, diluted to 10% solids with a buffer of pH 10, and treated with 1.0% chymotrypsin (Sigma C4129), on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 120 minute reaction time for the mixture. The pH of the reaction mixture was then adjusted to 7.5, after which the product was heat inactivated and freeze dried. The product properties are reported in Table 1.

## **EXAMPLE 13**

The jet cooked curd of Example 1 was adjusted to a pH value of 10 and treated with 1.5% papain, on a dry curd basis, as described in Example 1. The pH was maintained at a value of 10 during the 30 minute reaction time for the mixture. At the end of this time, the product was heat inactivated at pH 10 and spray dried as described in Example 1. The product properties are reported in Table 1.

# **EXAMPLE 14**

The room temperature curd of Example 1 was adjusted to a pH value of 10 and treated with 0. 75% papain, on a dry curd basis. As described in Example 1, the enzyme and sodium hydroxide were added in line in a continuous system. The pH was maintained at a value of 10 by the in line addition. The reaction took place in two tanks with a 15 minute residence time in the first tank and a 45 minute reaction time in the second tank. The pH of the reaction mixture was then adjusted in line to a value of 7. 5. The product was heat inactivated and spray dried as described in Example 1. The product properties are reported in Table 1.

#### **COMPARATIVE EXAMPLE 1**

For purposes of comparison, the jet cooked curd of Example 1 was adjusted to a pH value of 7 and treated with 1.0% bromelin. The pH was allowed to drop during hydrolysis. After 30 minutes of reaction the enzyme was heat inactivated and spray dried as described in Example 1. The product properties are reported in Table 1.

## COMPARATIVE EXAMPLE 2

For purposes of comparison, the room temperature curd of Example 1 was treated by a conventional process as described in U.S. patent 3,694,221. The curd, at 12% solids was adjusted to a pH value of 7.0 and jet cooked to 305°F (152°C) for 9 seconds, after which it was cooled to 120°F (49°C). The pH of the curd was then raised to a value of 8. 5 and the curd was treated with 1.0% alcalase, on a dry solids basis. The mixture was reacted for 120 minutes and then jet cooked to 305°F (152°C) for 9 seconds to inactivate the enzyme.

The results are shown in Table 1.

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TABLE 1: SUMMARY OF PROPERTIES

EXAMPLE	Enzyme	*a	TNBSb	Bitterness e
Number	Dea	midation		
1	Papain	26	<b>57</b>	1.3
2	Pancreatin	25	114	1.1
3	Alcalase	<b>28</b>	182	5.3
4	Papain	16	45	1. 3
5	Pancreatin	20	33	
6	Alcalase	28	164	
7	Papain	9	42	
8	Papain	14	-	-
9	Papain	16	-	-
10	Papain	18	73	-
1 1	Trypsin	14	29	-
1 2	Chymotrypsin	29	-	-
1 3	Papain	26	54	-
14	Papain	11	45	~
C. E. 1 *	Bromelain		60	7. 1
C. E. 2 *	Alcalase		196	<u>-</u>

<sup>\*</sup> Comparative Examples

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TABLE 1: SUMMARY OF PROPERTIES (continued)

EXA	MPLE	Enzyme	Solubility	(%)	C	MWD d	
Num	ber		pH 7.0	рН	5. 5	Mn	Mw
1		Papain	74		42	2615	16,052
2		Pancreati:			57	1204	8,000
3		Alcalase	88		88	1069	6,727
4		Papain	61		22	3349	22,650
5		Pancreati	n -		_	-	-
6		Alcalase	82		74	1909	10,333
7		Papain	-		-	-	-
8		Papain	-		-	-	-
9		Papain	_		-	-	-
10		Papain				2274	11,573
11		Trypsin	62		10	-	-
12		Chymotryps	sin -		-	-	-
13		Papain	96		41	-	-
14		Papain	68		24	-	-
C. E	. 1	Bromelain	37		28	2599	15,585
C. E	. 2	Alcalase	62		57	· _	-

- The diffusion method of Conway and O' Malley (J. Biochem, 36:655, 1942) is used to measure NH<sub>3</sub> released after a 1.25% product slurry in 3N HCl has been heated in a sealed vial for 3 hours at 110°C. The reference for zero percent deamidation is 18.3mg NH, per g of undeamidated protein.
- Moles end groups per  $10^5 \mathrm{g}$  of protein as measured by the method of R. Fields (Biochem, J., 124, 581, 1971). Unhydrolyzed soy proteins have average TNBS 50 of 30.

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- Method of J. L. Shen, Cereal Chem. 53: 902 (1976). . C
- Molecular weight distribution as determined by HPLC đ in 6M GuHCl and dithiothreitol using a TSK 2000 size exclusion column (Pharmacia, Pleasant Hill, CA). Mn = number average molecular weight in daltons. 10 Mw = weight average molecular weight in daltons.
- Bitterness scores from trained panel tasting 3% slurries in water at pH 7.0 and 122°F. 15 0 = least bitter 10 = most bitter.

As shown by Table 1, the process of the present invention enables the person skilled in the art to choose the desired combination and degree of characteristics of the resulting product. For example, the practitioner can choose, within limits, the degree of solubility and the degree of deamidation desired for a particular resulting product, as the end use requires.

The functional properties of representative products made by the process of the present invention are summarized in Tables 2 - 4.

TABLE 2

		ρHSo	lubility Profile :	
		% Prote	ein Soluble <sup>a</sup>	
ρН	Example 4	Example 2	Example 3	Comparative Example 1
2. 0	63%	83%	84%	19%
4. 0	22%	55%	80%	18%
4. 5	19%	55%	73%	21%
5. 0	22%	58%	82%	23%
6.0	35%	64%	86%	30%
7. 0	61%	79%	88%	37%
9.0	68%	87%	88%	38%

a Solubility method of J. L. Shen, Cereal Chem. 53: 902 (1976)

TABLE 3

Fo	am Density a	
Example 4	0. 092 g/cc	0. 099 g/cc
Example 2	0.061 g/cc	0.074 g/cc
Example 3	0. 065 g/cc	0.060 g/cc
Comp. Example 1	0. 084 g/cc	0. 085 g/cc

a The method for preparing the foam is as follows: Fifteen grams of product is slurried in 135 g of water and whipped with a wire whip for 6 minutes at highest speed (10) using a Kitchen Aid Model K5A mixer. After whipping, the density is measured with a 5 oz Solo cup.

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#### TABLE 4

Emulsion Cap	acity at pH 7ª
Product	Emulsion Capacity (ml oil/g Product)
Example 4 Example 2 Example 3 Comp. Example 1	131 98 52 113

a The method is as follows: 100 ml of a 0.5% product in water slurry is transferred to a 1 litre blender bowl. Soy bean oil at a constant flow rate of 49 ml/min is blended into the slurry with a Waring Blender (Model 70129) at 15,300 rpm until a phase inversion from oil in water emulsion to water in oil emulsion occurs. The end point is detected by a loss in conductance. The emulsion capacity is calculated after subtracting out a blank value for water.

#### Claims

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- 1. A method of treating a protein material having an insoluble fraction, which comprises subjecting an aqueous slurry of the protein material to controlled hydrolysis and deamidation, the controlled hydrolysis and deamidation including adjusting the slurry to an elevated pH, adding a proteolytic enzyme to the slurry and reacting the slurry for a time and at a temperature which, in combination with the pH level and enzyme, are effective partially to hydrolyse and partially to deamidate the protein, the treatment being effective to substantially uniformly hydrolyse and deamidate across substantially the entire protein substrate including the insoluble fraction of the protein substrate, thereby increasing the solubility of the protein and increasing the functional properties of the protein without producing a bitter flavour in the treated protein.
- 2. A process according to claim 1, in which the enzyme is an animal, plant or microbial enzyme.
- A Process according to claim 1 or claim 2, in which the pH of the slurry is adjusted to a value of from 9
  to 11.
  - 4. A process according to claim 3, in which the pH is adjusted to a value of about 10.
- A process according to any one of claims 1 to 4, in which the treated slurry is reacted at a temperature of from 10 to 75°C.
- 6. A process according to any one of claims 1 to 5, in which the treated slurry is reacted for a period of from 10 to 240 minutes.
  - 7. A process according to any one of claims 1 to 6, in which the reacted slurry is subsequently heated to inactivate the enzyme and stop the reaction.
- 8. A process according to any one of claims 1 to 7, in which the enzyme is added at a level of from about 0.01 to about 5% by weight based on the dry weight of the protein in the slurry.
  - 9. A process according to any one of claims 1 to 8, in which the protein is deamidated to between about 5 to 48%.
- 10. A process according to any one of claims 1 to 9, in which the reaction is effected under such conditions that the treated protein is rendered between from 60 to 100% soluble in a pH 7 aqueous solution.

- 11. A process according to claim 10, in which the solubility of the protein is above 80%.
- 12. A process according to claim 10, in which the solubility of the protein is above 90%.
- 5 13. A process according to any one of claims 1 to 12, in which the treated protein is subsequently dried.
  - 14. A process according to claim 13, in which the treated protein is spray dried.
- 15. A process according to any one of claims 1 to 14, in which the treated protein has a molecular weight (Mn) of from 800 to 4000.
  - **16.** A process according to any one of claims 1 to 15, in which the protein material is a vegetable protein material.
- 15. A process according to claim 15, in which the protein material is an oilseed protein material.
  - 18. A process according to claim 16, in which the protein material is a soy protein material, preferably an isolated soy protein.
- 19. A process according to any one of claims 1 to 18, in which the treatment is effected under conditions of time, temperature and pH such as to hydrolise and deamidate, at least partially, core proteins of the protein material.
- 20. A method of treating an isolated vegetable protein material having an insoluble protein core or globular fraction comprising forming an aqueous slurry of the isolated vegetable protein material and subjecting 25 the slurried protein to a controlled hydrolysis and deamidation, the treatment including adjusting the pH of the slurried protein material to between about 9 and 11, adding a proteolytic enzyme to the slurried protein material, the enzyme being added at a level of between about 0.01 and 5% by weight of the dry protein in the slurry, and reacting the treated slurry at a temperature of between about 10 and 75°C for a time of between about 10 and 240 minutes, the combined treatment being effective to partially 30 hydrolyse and partially deamidate the protein, the combined treatment being further effective to substantially uniformly hydrolyse and deamidate the protein across substantially the entire protein substrate, including the insoluble fraction of the substrate, without excessively hydrolysing the soluble fraction of the substrate, and to produce a treated protein having a molecular weight distribution (Mn) between about 800 and 4000, the treated protein being deamidated at a level of between about 5 to 35 48% and having a solubility in a pH 7 aqueous solution of between about 60 and 100%, the controlled hydrolysis and deamidation being effective to substantially increase the solubility of the protein and increase the functional properties of the protein without producing a bitter flavour in the treated protein.
- 21. A process according to claim 20, which also incorporates the features of any one of claims 2 to 18.

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# EUROPEAN SEARCH REPORT

Application Number

EP 90 31 1110

	OCUMENTS CONS				
ategory		ith indication, where appropriate, evant passages		Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. CI.5)
x	FR-A-2 338 001 (NOUD II * Claims 1-7,13-14; example			-6,8, 5-19	A 23 J 3/34
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X	FR-A-2 186 193 (THE QU * Page 6, lines 5-36; examp		1	-6,15-19	
X	WPIL / DERWENT, access lications Ltd, London, GB; & JP-A-63 036 797 (FUJI C * Abstract *		1	-5,7,13, 5-19	
Α	IDEM		9	,20	
Y	US-A-4 757 007 (MASAAI * Example 1; column 2, line		7	,10-13	
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	The Hague	13 June 91			SANTOS Y DIAZ A.I.
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